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(54) Title: METHODS USEFUL IN ENDOTOXIN PROPHYLAXIS AND THERAPY

(57) Abstract

Treatment and prophylaxis of endotoxin caused toxicity is disclosed. This is accomplished by administering a lipid into which the endotoxin of concern is associated preferably together with a peptide which is not an apolipoprotein. Preferably, the two components are administered in the form of a reconstituted particle, although this is by no means required.

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METHODS USEFUL IN ENDOTOXIN PROPHYLAXIS AND THERAPY

RELATED APPLICATION

This application is a continuation-in-part of U.S. Patent Application Serial Number 07/928,930 filed August 12, 1992.

FIELD OF THE INVENTION

This invention relates to the treatment of endotoxin poisoning. More particularly, it relates to the treatment of such poisoning via administration of various compositions which act to neutralize and/or remove endotoxins from the organism.

BACKGROUND AND PRIOR ART

Normal serum contains a number of lipoprotein particles which are characterized according to their density, namely, chylomicrons, VLDL, LDL and HDL. They are composed of free and esterified cholesterol, triglycerides, phospholipids, several other minor lipid components, and protein. Very low density lipoprotein (VLDL) transports energy, in the form of triglycerides, to the cells of the body for storage and use. As triglycerides are delivered, VLDL is converted to low density lipoprotein (LDL). Low density lipoprotein (LDL) transports cholesterol and other lipid soluble materials to the cells in the body, while high density lipoprotein (HDL) transports excess or unusable to the liver for elimination. Normally, these lipoproteins are in balance, ensuring proper delivery and removal of a lipid soluble materials. Abnormally low HDL can cause a number of diseased states as well as constitute a secondary complication in others.

Under normal conditions, a natural HDL is a solid particle with its surface covered by a phospholipid monolayer that encloses a hydrophobic core. Apolipoprotein A-I and A-II attach to the surface by interaction of the hydrophobic face of their alpha helical domains. In its

nascent or newly secreted, form the particle is disk-shaped and accepts free cholesterol into its bilayer. Cholesterol is esterified by the action of lecithin:cholesterol acyltransferase (LCAT) and is moved into the center of the disk. The movement of cholesterol ester to the center is the result of space and solubility limitations within the bilayer. The HDL particle "inflates" to a spheroidal particle as more and more cholesterol is esterified and moved to the center. Cholesterol ester and other water insoluble lipids which collect in the "inflated core" of the HDL are then cleared by the liver.

Jonas et al., Meth. Enzym. 128A: 553-582 (1986) have produced a wide variety of reconstituted particles resembling HDL. The technique involves the isolation and dilapidation of HDL by standard methods (Hatch et al., Adv. Lip. Res. 6: 1-68 (1968); Scanu et al., Anal. Biochem. 44: 576-588 (1971) to obtain apo-HDL proteins. The apoproteins are fractionated and reconstituted with phospholipid and with or without cholesterol using detergent dialysis.

Matz et al., J. Biol. Chem. 257(8): 4535-4540 (1982) describe a micelle of phosphatidylcholine, with apoliprotein A1. Various ratios of the two components are described, and it is suggested that the described method can be used to make other micelles. It is suggested as well to use the micelles as an enzyme substrate, or as a model for the HDL molecule. This paper does not, however discuss application of the micelles to cholesterol removal, nor does it give any suggestions as to diagnostic or therapeutic use.

Williams et al., Biochem. & Biophys. Acta 875: 183-194 (1986) teach phospholipid liposomes introduced to plasma which pick up apoproteins and cholesterol. Liposomes are disclosed, which pick up apoprotein in vivo, as well as cholesterol, and it is suggested that the uptake of cholesterol is enhanced in phospholipid liposomes which have interacted with, and picked up apoproteins.

Williams et al., Persp. Biol. & Med. 27(3): 417-431 1984) discuss lecithin liposomes as removing cholesterol. The paper summarizes earlier work showing that liposomes which contain apoproteins remove cholesterol form cells in vitro more effectively than liposomes which do not contain it. They do not discuss in vivo use of apoprotein containing liposomes or micelles, and counsel caution in any in vivo work with liposomes.

It is important to note that there is a clear and significant difference between the particles of the present invention, and the liposomes and micelles described in the prior art. The latter involve a bilayer structure of lipid-containing molecules, surrounding an internal space. The construction of liposomes and micelles precludes filling the internal space, however, and any molecular uptake is limited to the space defined between the two lipid layers. As a result, there is much less volume available for pick up and discharge of materials such as cholesterol and other lipid soluble materials than there is for the particles of this invention, which expand in a fashion similar to a balloon, with interior space filling with the material of choice.

Anantharamaiah, in Segrest et al., Meth. Enzymol. 128: 627-647 (1986) describe a series of peptides which form "helical wheels", as a result of the interaction of the amino acids in the peptide with each other. Such helical wheels present a nonpolar face, and a polar face in their configuration.

Endotoxic shock is a condition, often fatal, provoked by the outer membrane of most gram negative bacterial (e.g., Escherichia coli; Salmonella tymphimurium). The structure of the bacterial outer membrane has been fairly well elucidated, and a unique molecule, referred to as lipid A, which is linked to acyl chains via lipid A molecule's glucosamine backbone. See Raetz, Ann. Rev. Biochem. 59: 129-170 (1990) in this regard.

The lipid A molecule serves as membrane anchor of a

lipopolysaccharide structure ("LPS") and it is the LPS which s implicated in the development of endotoxic shock. It should be pointed out that LPS molecules are characterized by a lipid A type structure and a polysaccharide portion. This latter moiety may vary in molecular details in different LPS molecules, but it will retain the general structural motifs characteristic of endotoxins. It would be incorrect to say that the LPS molecule is the same from bacteria to bacteria (see Raetz, supra). It is common in the art to refer to the various LPS molecules as "endotoxins", and this term will be used hereafter to refer to LPS molecules collectively.

In U.S. Patent No. 5,128,318 the disclosure of which is incorporated by reference, it was taught that reconstituted particles containing both an HDL associated apolipoprotein and a lipid capable of binding an endotoxin to inactivate it could be used as effective materials for alleviating endotoxin caused toxicity.

It has now been found that various other materials may be used to treat endotoxin caused toxicity. Specifically, it has been found that apolipoproteins are not required in reconstituted particles, and that the reconstituted particle may contain a peptide and a lipid as defined supra, wherein the peptide is not an apolipoprotein.

It has also been found that endotoxin caused toxicity may be treated via sequential administration of either an apolipoprotein or a peptide followed by a lipid as described supra. It appears that following sequential administration the components assemble as a reconstituted particle and then act to remove endotoxin.

It has also been found that at least some individuals possess native levels of apoliprotein which are higher than normal levels such that effective endotoxemia therapy may be effectuated by administering reconstituted particles containing no apolipoprotein or peptide, but containing the lipid described supra.

In addition, the invention involves the use of the reconstituted particles and the components discussed herein for prophylaxis against endotoxin caused toxicity, by administering prophylactically effective amounts to subjects in need of prophylaxis.

Such subjects include patients suffering from infections or receoving from surgery. These patients sometimes exhibit drops in plasma HDL levels, to 5-30% of normal levels. It is highly desirable, in these cases, for early prophylasix with HDL, so as to compensate for these drops.

These and other aspects of the invention are described in the disclosure which follows.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows how reconstituted particles containing Apo-A1, phospholipid and cholate form.

Figure 2 shows the reception of LPS molecules by reconstituted particles.

Figure 3 shows experiments in which a peptide in accordance with the invention as used to study reduction of endotoxin caused toxicity in a mouse model.

Figure 4 shows the formation of helical wheels by various peptides.

DETAILED DESCRIPTION OF THE EMBODIMENTS

Example 1

Studies were carried out to determine the survival rate of mice challenged with S. tymphimurium endotoxin. Outbred male, Swiss-Webster mice received either saline (20 mice), reconstituted HDL particles (40 mice), or reconstituted peptide 18A (20 mice), via injection through the tail vein. The particulars of the injection materials are as follows:

a. HDL particles

Particles were prepared from apo-Hu-HDL (85%-AI; 15% AII and apo C), reconstituted with 95% pure egg phosphatidylcholine (2:1 W/W), using detergent dialysis, in accordance with Matz et al., J. Biol. Chem. 257: 4535-4540 (1982), and U.S. Patent No. 5,128,318, the disclosure of which is incorporated by reference.

b. peptide particles

The peptide 18A has the amino acids sequence:

Asp-Trp-Leu-Lys-Ala-Phe-Tyr-Asp-Lys-Val-Ala-Gly-Lys-Leu-Lys-Glu-Ala-Phe

Samples of peptide were also mixed and reconstituted with 95% pure eggs phosphatidylcholine as per Matz et al., supra (2:1 w/w), and U.S. Patent No. 5,128,318 also using detergent dialysis. The resulting particles are identical to those disclosed in U.S. Patent No. 5,128,318 except that a peptide component was present, rather than the apo-HDL of the Matz and patent references.

Within fifteen minutes of administration of the reconstituted material, the mice were administered, intraperitoneally, 10 mg/kg body weight of Salmonella LPS. The criterion for evaluation was survival. Figure 3 presents these results, and indicates nearly 4 fold superiority over the saline control. The synthetic peptide is almost as effective as the reconstituted apo-HDL containing particles.

The examples infra show the efficacy of various treatment methodologies for alleviating endotoxin caused toxicity. The feature which unites all forms of therapy described herein is the need for both a peptide or an apolipoprotein to be present, and a lipid in which the endotoxin causative agent associates. "Associates", as the term is used herein refers to the interaction of lipid and the lipid portion of the endotoxin molecule. The interaction permits removal of the endotoxin by the particle structure to a clearing site in the body,

particularly the liver. Figure 2 shows the mechanism of association.

The therapeutic efficacy attained with the invention described herein also suggests adaptation of the methodology for prophylaxis against endotoxemia. There are very well recognized situations where individuals are put at risk for exposure to endotoxins or infections in which endotoxins are implicated. including, but not limited to surgery, treatment of wounds, burns, immunosuppression, and so forth. The invention encompasses prophylactic methodologies wherein an individual at risk for endotoxemia receives an effective amount of the described materials sufficient to prevent or to lower the risk of endotoxemia. The various situations in which an individual is exposed to endotoxins are well known to the skilled artisan and need not be repeated here.

The therapeutic regime described supra involves the administration of reconstituted particles containing peptide and lipid. It is also possible to administer the components of the particles separately - i.e., individual doses of the peptide and the lipid, or one of each. The dosing may be sequential or simultaneous. It is also within the scope of the invention to treat some patients by administering only the lipid component or only the peptide component. Such patients will be those who show either a level of apolipoproteins sufficiently high in their blood or plasma such that the artisan will expect in vivo formation of particles such as those described herein, where native apolipoprotein combines with the administered lipid to form the particles which remove the LPS, or those who are hyperlipedemic and thus do require only the peptide to form the requisite construct.

Both the treatment and prophylactic therapies described herein may be carried out in this way.

It is preferred that the peptides of the invention be amphipathic, such that when placed in polar solutions the peptides take on a configuration wherein hydrophobic amino

acids cluster preferentially on one face and hydrophilic amino acids cluster on the other. Various three dimensional structures may result, one of which is the helical wheel configuration of the peptides presented in figure 4, which are described in Anantharamaiah supra, the disclosure of which is incorporated by reference. Peptide 18A is an especially preferred peptide for use in accordance with the invention.

The lipid to be used in any of the forms of therapy described herein may vary, with phospholipids, and especially phosphatidylcholine being preferred. There are a large number of different endotoxins known to the skilled artisan, and it is only necessary that the lipid be one with which the endotoxin may associate.

The demonstrated efficacy of the invention, as elaborated upon supra, suggests extension to other therapeutic situations. For example, there are situations where an infection by, e.g., bacteria, is treated with a bacteriocidic drug, leading to death of the pathogenic organism, but also to production or release of toxins thereby. A classic example of this situation is the treatment of meningitis, be it viral or bacterial. Following treatment with antibiotics, meningitis bacteria die, and release toxins into, e.g., the cerebrospinal fluid. These toxins pose a great clinical threat to patient. Administration of the particles of the invention, which have been shown to be effective agents in endotoxin therapy, should serve to eliminate the toxins at issue.

Another issue related to meningitis causing bacteria, and bacteria in general, is resistance of the pathogen to drugs, such as antibiotics. While there are many theories and reasons for development of drug resistance in microorganisms, the fact remains that for any drug to be bacteriocidal it must cross the cell membrane of the targeted organism. It is well known that cell membranes are composed, inter alia of lipids and proteins. Fusion of the particles of the invention, which are also composed

of lipid and protein, would be expected to change the permeability, fluidity, and other properties of the microorganism, thereby leading to reduced drug resistance, susceptibility to other drugs, or both. This is not only true of meningitis related bacteria, but applies to bacteria in general, be they gram negative or gram positive. Parasites and other pathogens possessing cell membranes should behave in the same way. Examples of bacteria and parasites which can be so treated include pneumococcus, M. tuberculosis, and so forth. Conditions such as leprosy may be treated in the manner described herein, the treatment being useful against Mycobaterium leprae and other Mycobacteria (e.g., M. avium). The mechanisms by which the pathogens are eliminated include the antibiotic resistance decrease set forth supra, as well as fundamental changes in the membrane (e.g., removal of membrane associated materials such as glycoproteins, exposure of other unavailable or inaccessible epitopes, so as to enhance immunotherapy; modification of the membrane such that attachment to a host cell target is impeded or blocked, and/or modification of the membrane such that growth or reproduction of the organism in question is impeded or blocked).

In the case of viral infections, the coat of many viruses is known to have a lipid component. Use of the particles of the invention "dissolves" the coat, either rendering the virus vulnerable to immune attack, or to removal from the art.

On a related, but somewhat different level, the particles of the invention may be used to treat various conditions not directly linked to infection. For example, many pathological conditions include, as one of their manifestations, changes in the cell membrane of effected cells. Cancer is the best known, but certainly not the only example of this phenomenon. Fusion of the particles of the invention to cancer cells would result in changes similar to those outlined supra for bacteria and other

infectious agents, again leading to enhanced susceptibility to therapeutic agents and reduced resistance to others. Also, it is known that, in the course of chemotherapy, cytokines are produced by the treated cells, which then provoke inflammation in the subject. Treatment with the particles may lead to reduction or elimination of cytokine production by the transformed cells, and thereby serve as an inflammation therapy.

In connection with the applicability of the particles of the invention to inflammation therapy, treatment by administration to a pertinent tissue or organ is contemplated. One example of the applicability of the invention is to inflammation in the eye. For various reasons well known to the artisan, damage to the eye generally extends well beyond the initial provocation. Again, endotoxins are associated with this "extended damage", for all of the reasons set forth supra. Administration of the particles of the invention in a manner which targets these directly to the eye (e.g., in a lens implant, eyewash, etc.), would be expected to lead to the binding and neutralization of toxins associated with the eye damage, be these bacterial toxins such as endotoxins, or others. On a different level, ocular therapy with the particles of the invention would be expected to lead to changes in phospholipid and phospholipid fatty acid composition in the cells, in turn leading to control of second messenger production (e.g., Phosphatidyl inositol).

It should not be assumed that inflammation therapy in accordance with the invention is limited to the eye. Another example of targeted inflammo-therapy involves treatment of the lungs. In those cases where lung disease results from an infectious organism, including Nocardia, Pneumocystis carinii, and so forth, the particles of the invention can be used in the same manner they are used in treatment of the eye - i.e. - the particles can be directed to the tissue in the form, e.g., of an aerosol administered by a bronchodilator or by some other means, at which point

they function in the same way the particles do in the eye. In addition, the recognized ability of the particles to interact with target molecules makes them useful in treatment of the lungs in at least two other ways. Neutrophils are attracted to lungs and become active to both adult respiratory distress syndrome and emphysema. Destruction of elastin by proteases, typified by neutrophil elastase is a pathogenic mechanism common to both diseases. This leads to lung fibrosis caused by deposition of amyloid-A in the lungs. This, in turn, leads to attraction of neutrophils and their activation, including the release of elastase. The particles can bind to serum amyloid-A, thereby preventing its deposition in lung tissue, thereby "short circuiting" the mechanism of action described supra. Further, as the particles de facto become a competitive substrate for amyloid-A, the release of neutrophil elastase is directly inhibited.

Inhibition of elastase can also lead to therapy of arthritis, via use of the particles of the invention. In at least two forms of arthritis, i.e., autoimmune and septic arthritis, mechanisms are involved which can be treated using the invention. In the case of autoimmune arthritis, the synovial linings of the joints are inflamed, the inflammation being mediated by lymphokines. Septic arthritis is not unlike other endotoxin related conditions, in that the endotoxins mediate the inflammation of the synovial linings. As has been described, supra, the particles of the invention bind to and neutralize the recited inflammation mediators. Also, a mechanism recited supra for lung therapy, i.e., the inhibition of elastase, may be involved in arthritis as well, both in connection with specific inhibition of elastase, as well as by inhibition of other neutrophil proteases, it being well known that neutrophils are found at the sites of inflammation. Further, the particles may directly effect lymphokine and cytokine inflammation mediators.

The foregoing disclosure sets forth one methodology

for preparing the particles of the invention, but other methodologies are equally applicable. For example, one may dissolve the lipid of interest in a solvent, such as sodium cholate/sterile intravenous saline, followed by mixing with the peptide of interest. Cholate is then removed and recombinant particles are prepared in accordance with, e.g. Bonomo et al, J. Lipid. Res 29: 380-384 (1988).

Peptides may be prepared in any of the standard methodologies known to the skilled artisan, excluding solid phase synthesis, expression of recombinant DNA as well as proteolytic cleavage followed by purification, and so forth. The first stated methodology is preferred, in view of the control it affords to the investigator.

The skilled artisan will be aware of various peptides, lipids and endotoxins useful in the invention as described therein, and all are encompassed by applicants' invention.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

SEQUENCE LISTING

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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Asp Trp Leu Lys Ala Phe Tyr Asp Lys Val Ala Glu Lys Leu Lys
5 10 15
Glu Ala Phe

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Lys Trp Leu Asp Ala Phe Tyr Lys Asp Val Ala Lys Glu Leu Glu
5 10 15
Lys Ala Phe

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Asp Trp Leu Lys Ala Phe Tyr Asp Lys Ala Glu Lys Leu Lys Glu
5 10 15
Ala Phe

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Pro Lys Leu Glu Glu Leu Lys Glu Lys Leu Lys Glu Leu Leu Glu
5 10 15
Lys Leu Lys Glu Lys Leu Ala
20

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Val Ser Ser Leu Lys Glu Tyr Trp Ser Ser Leu Lys Glu Ser Phe
5 10 15
Ser

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Val Ser Ser Leu Leu Ser Ser Leu Lys Glu Tyr Trp Ser Ser Leu
5 10 15
Lys Glu Ser Leu Ser
20

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Val Ser Ser Leu Leu Ser Ser Leu Leu Ser Ser Leu Lys Glu Tyr
5 10 15
Trp Ser Ser Leu Lys Glu Ser Glu Ser
20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Glu Leu Glu
5 10 15
Ala Leu Lys Gln Lys Met Lys
20

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Pro Leu Ala Glu Asp Leu Gln Thr Lys Leu Asn Glu Asn Val Glu
5 10 15
Asp Leu Arg Lys Gln Leu Val
20

We claim:

1. Method of treating a subject for endotoxin caused toxicity comprising administering to said subject an amount of a particle containing (i) a peptide which is not an apolipoprotein and (ii) a lipid with which said endotoxin causing said toxicity associates, in an amount sufficient to alleviate toxicity caused by said endotoxin.

2. Method of claim 1, wherein said peptide is amphipathic.

3. Method of claim 2, wherein said amphipathic peptide forms a helical wheel.

4. Method of claim 3, wherein said helical wheel forming peptide is a peptide selected from the group consisting of the peptides of figure 4.

5. Method of claim 4, wherein said peptide is peptide 18A.

6. Method of claim 1, wherein said lipid is a phosphatidylcholine.

7. Method of claim 1, wherein said endotoxin is an E. coli endotoxin.

8. Method of claim 1, wherein said endotoxin is an S. tymphimurium endotoxin.

9. Method for treating a subject for endotoxin caused toxicity comprising administering to said subject, in consecutive administrations, amounts of (i) a peptide which is not an apolipoprotein and (ii) a lipid with which said endotoxin causing said toxicity associates,

sufficient to alleviate toxicity caused by said endotoxin.

10. Method of claim 9, wherein said peptide is amphipathic.

11. Method of claim 10, wherein said amphipathic peptide forms a helical wheel.

12. Method of claim 11, wherein said helical wheel forming peptide is a peptide selected from the group consisting of the peptides of figure 4.

13. Method of claim 12, wherein said peptide is peptide 18A.

14. Method of claim 9, wherein said lipid is phophatidylcholine.

15. Method of claim 9, wherein said endotoxin is an E. coli endotoxin.

16. Method of claim 9, wherein said endotoxin is an S. tymphimurium endotoxin.

17. Method for treating a subject for endotoxin caused toxicity, comprising administering to said subject an amount of an endotoxin associating lipid sufficient to combine with native apolipoproteins in said subject and to alleviate toxicity caused by said endotoxin.

18. Method of claim 17, wherein said lipid is phosphatidyl- choline.

19. Method of claim 17, wherein said endotoxin is an E. coli endotoxin.

20. Method of claim 17, wherein said endotoxin is an S. tymphimurium endotoxin.

21. Method for treating a hyperlipodemic subject for endotoxin caused toxicity, comprising administering to said subject an amount of a peptide sufficient to combine with native lipid in said hyperlipidemic subject and to alleviate toxicity caused by said endotoxin.

22. Method of claim 21, wherein said peptide is peptide 18A.

23. Method of claim 21, wherein said endotoxin is an E. Coli endotoxin.

24. Method of claim 21, wherein said endotoxin is an S. tymphimurium endotoxin.

25. Method for reducing risk of endotoxin caused toxicity in a subject comprising administering to a subject prior to exposure to an endotoxin an amount of a particle containing (i) a peptide which is not an apolipoprotein, and (ii) a lipid with which said endotoxin associates, in an amount sufficient to reduce risk of said endotoxin caused toxicity in said subject.

26. Method for reducing risk of endotoxin caused toxicity in a subject, comprising administering to a subject prior to exposure to an endotoxin amounts of (i) a peptide which is not an apolipoprotein, and (ii) a lipid with which said endotoxin associates, in an amount sufficient to reduce risk of said endotoxin caused toxicity in said subject.

27. Method for reducing risk of endotoxin caused toxicity in a subject, comprising administering to a subject prior to exposure to an endotoxin an amount of a lipid with which said endotoxin associates sufficient to combine with native apolipoproteins in said subject and to reduce risk of endotoxin caused toxicity in said subject.

28. A particle containing (i) a peptide which is not an apoliprotein and (ii) a lipid which associates with an endotoxin which causes endotoxin-caused toxicity for use as an active therapeutic agent.

29. Use of a particle containing (i) a peptide which is not an apoliprotein and (ii) a lipid which associates with an endotoxin which causes endotoxin-caused toxicity in the manufacture of a medicament for the treatment of endotoxin-caused toxicity.

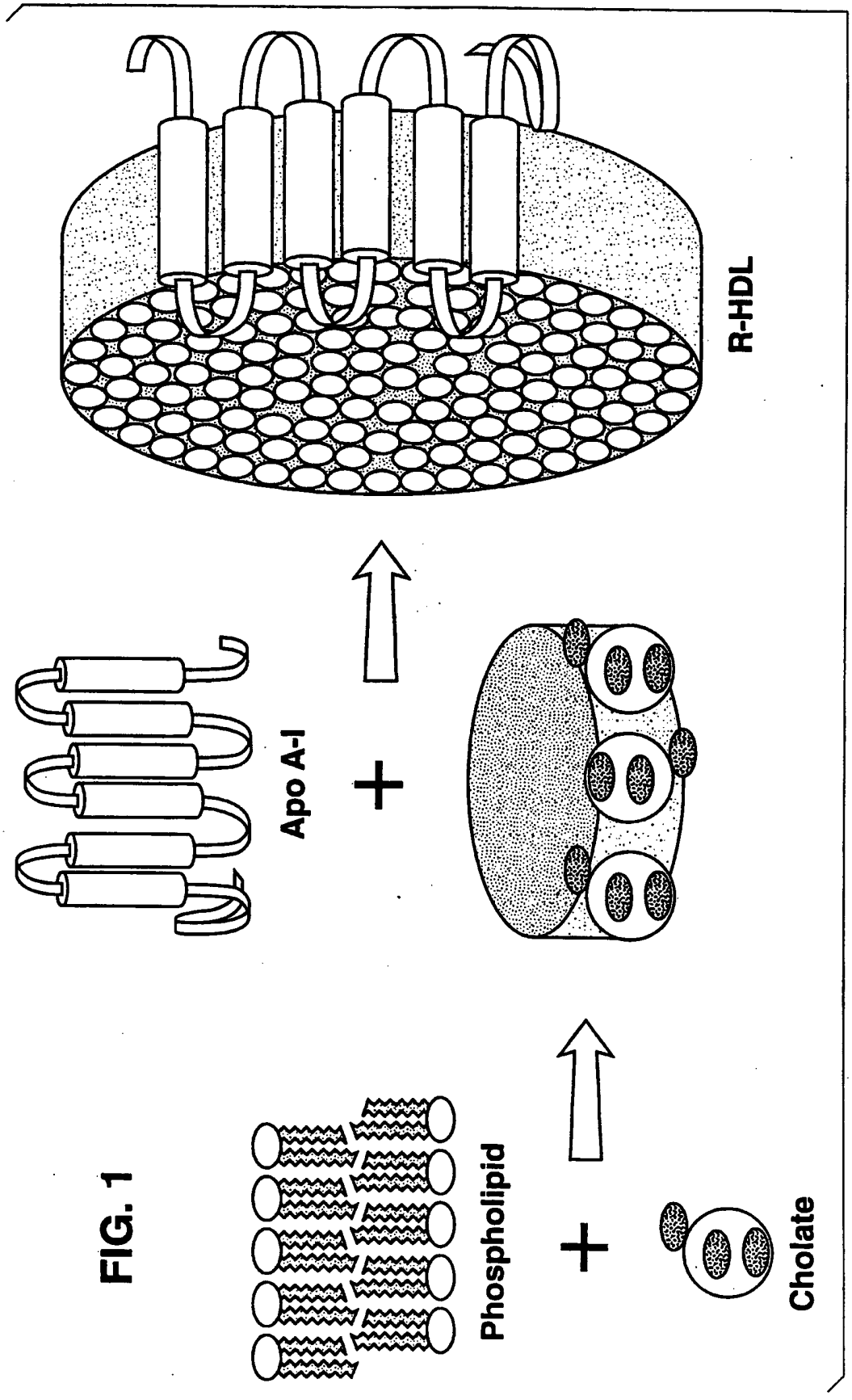


FIG. 1

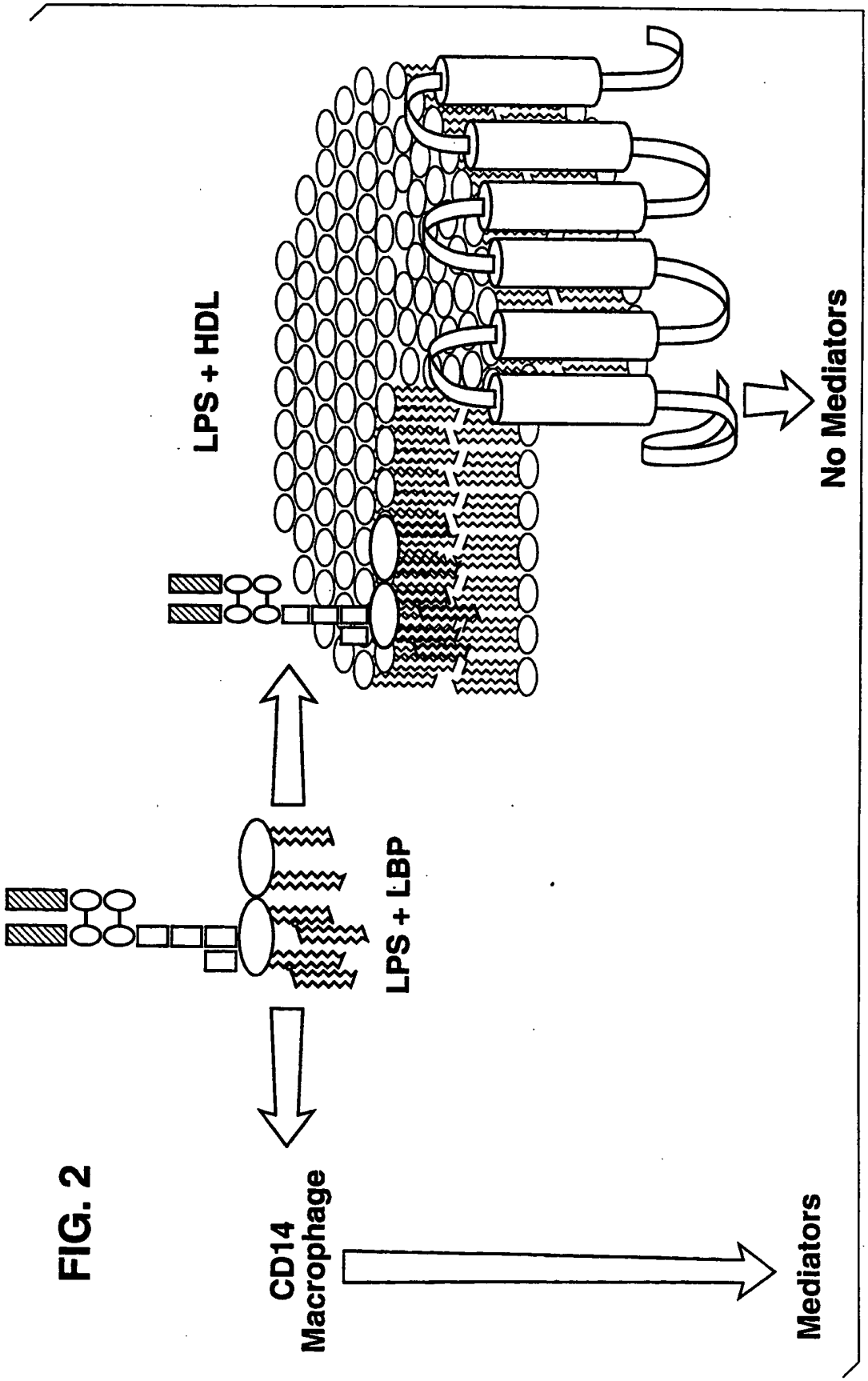


FIG. 2

FIG. 3

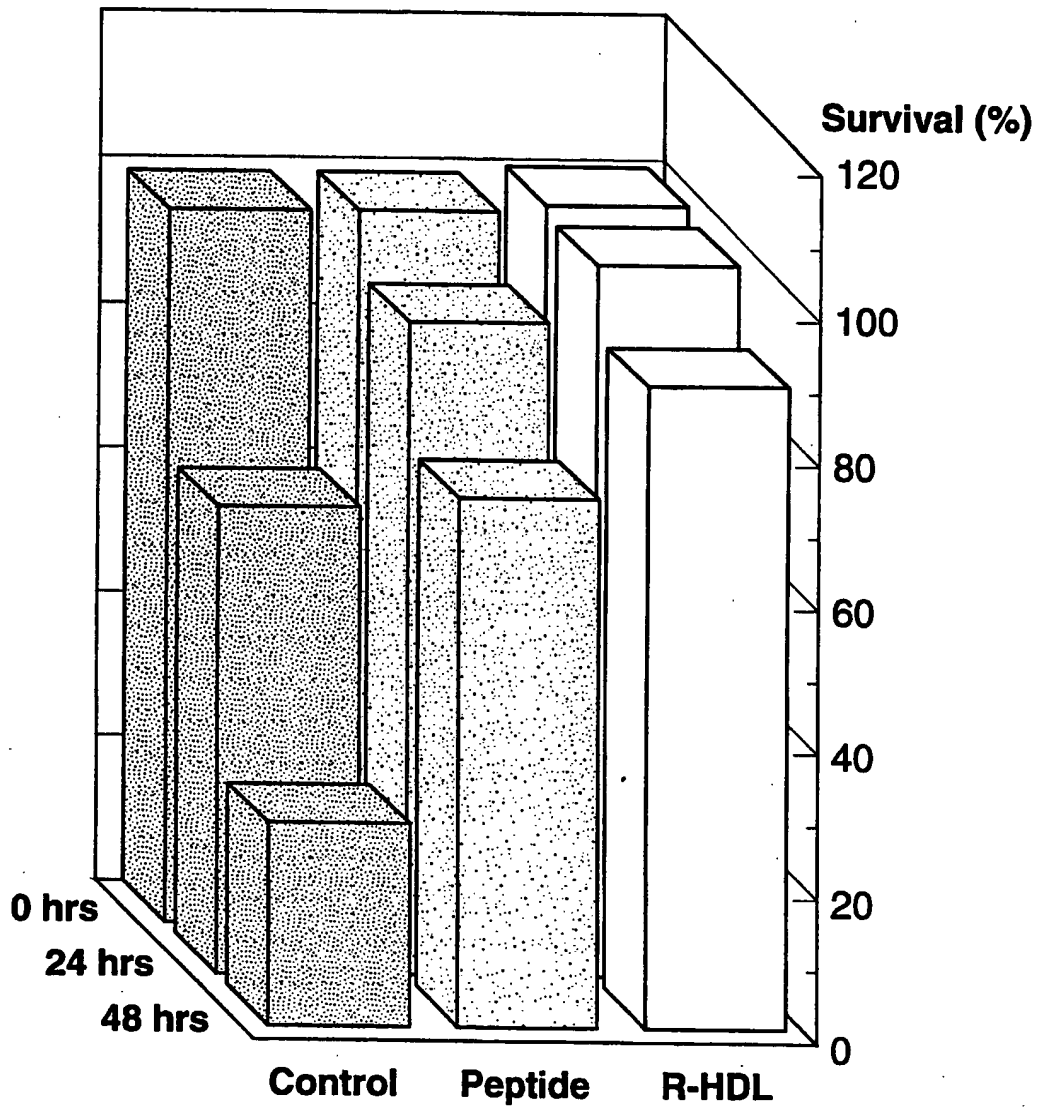

Survival (%)
120
100
80
60
40
20
0
0 hrs
24 hrs
48 hrs
Control
Peptide
R-HDL

FIG. 4A

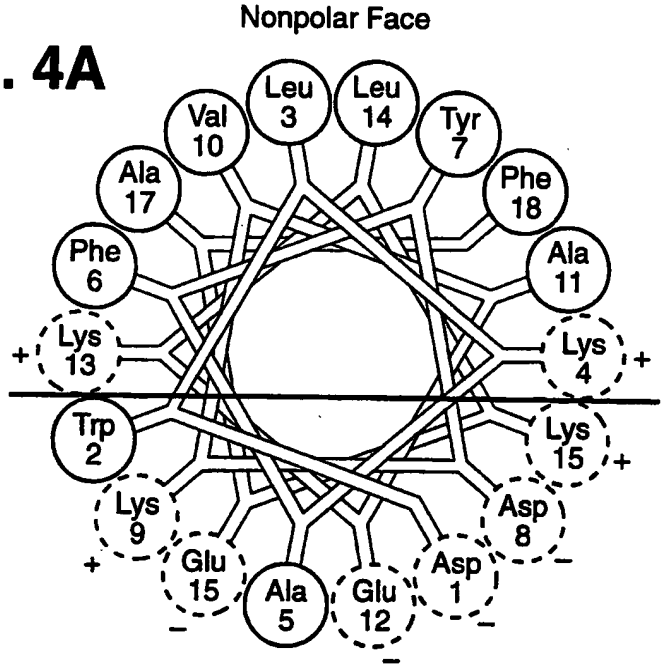

Nonpolar Face
Leu 3
Leu 14
Tyr 7
Phe 18
Ala 11
Lys 4
Lys 15
Asp 8
Asp 1
Glu 12
Ala 5
Glu 15
Lys 9
Trp 2
Phe 6
Ala 17
Val 10
Lys 13
Polar Face
18A

FIG. 4B

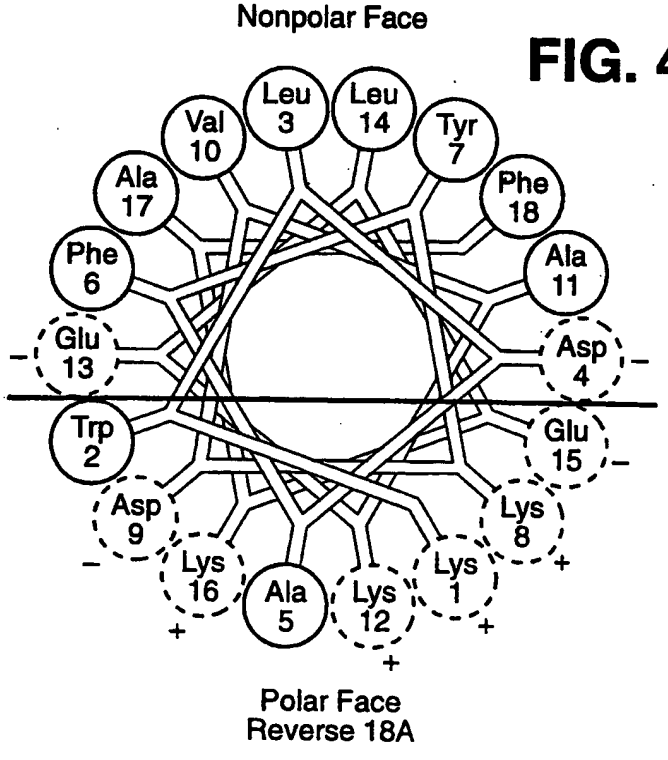

Nonpolar Face
Leu 3
Leu 14
Tyr 7
Phe 18
Ala 11
Asp 4
Glu 15
Lys 8
Lys 1
Lys 12
Ala 5
Lys 16
Asp 9
Trp 2
Phe 6
Ala 17
Val 10
Glu 13
Polar Face
Reverse 18A

FIG. 4C

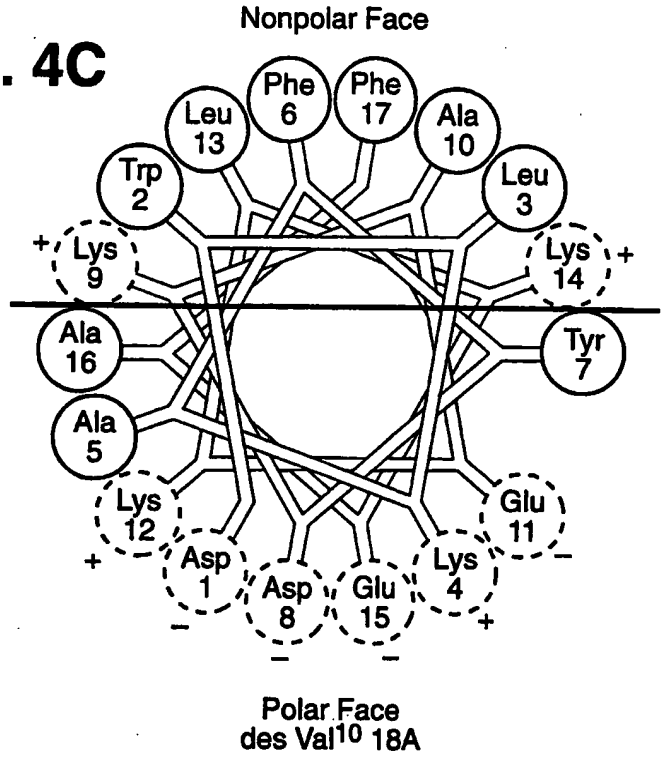

Nonpolar Face
Phe 6
Phe 17
Leu 13
Ala 10
Trp 2
Leu 3
Lys 9
Lys 14
Ala 16
Tyr 7
Ala 5
Lys 12
Glu 11
Asp 1
Asp 8
Glu 15
Lys 4
Polar Face
des Val10 18A

FIG. 4D

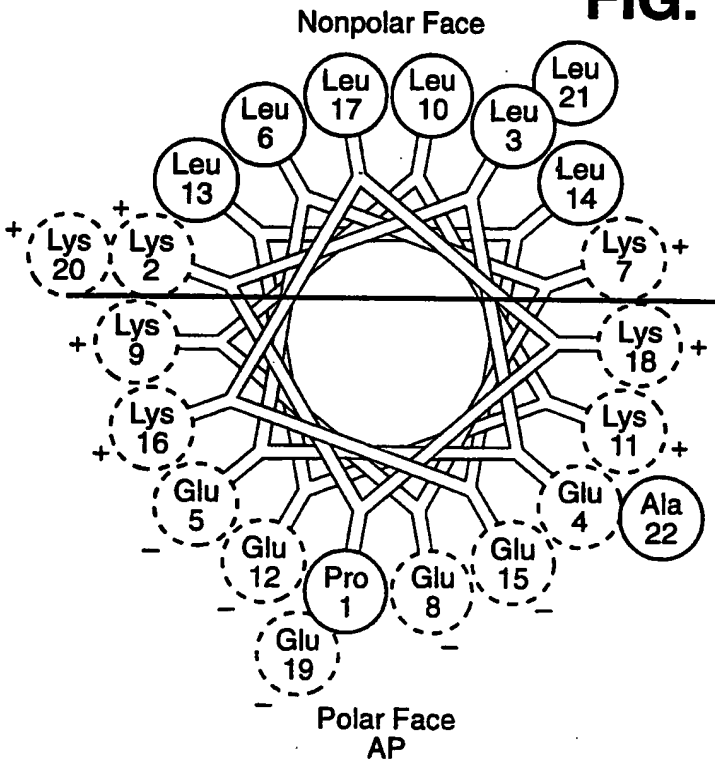

Nonpolar Face
Leu 17
Leu 10
Leu 21
Leu 6
Leu 3
Leu 13
Leu 14
Lys 20
Lys 2
Lys 7
Lys 9
Lys 18
Lys 16
Lys 11
Glu 5
Glu 4
Ala 22
Glu 12
Pro 1
Glu 8
Glu 15
Glu 19
Polar Face
AP

FIG. 4E

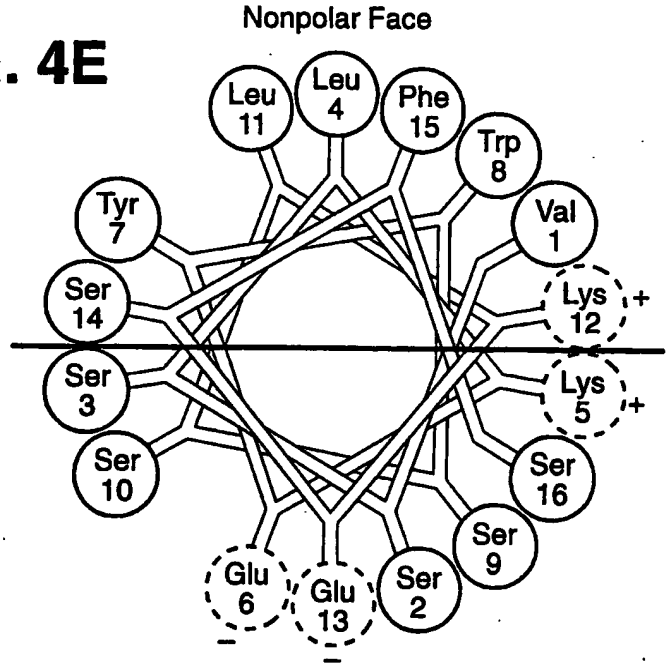

Nonpolar Face
Leu 11
Leu 4
Phe 15
Trp 8
Val 1
Tyr 7
Ser 14
Lys 12
+
Ser 3
Lys 5
+
Ser 10
Ser 16
Ser 9
Glu 6
Glu 13
Ser 2
−
−
Polar Face
LAP–16

FIG. 4F

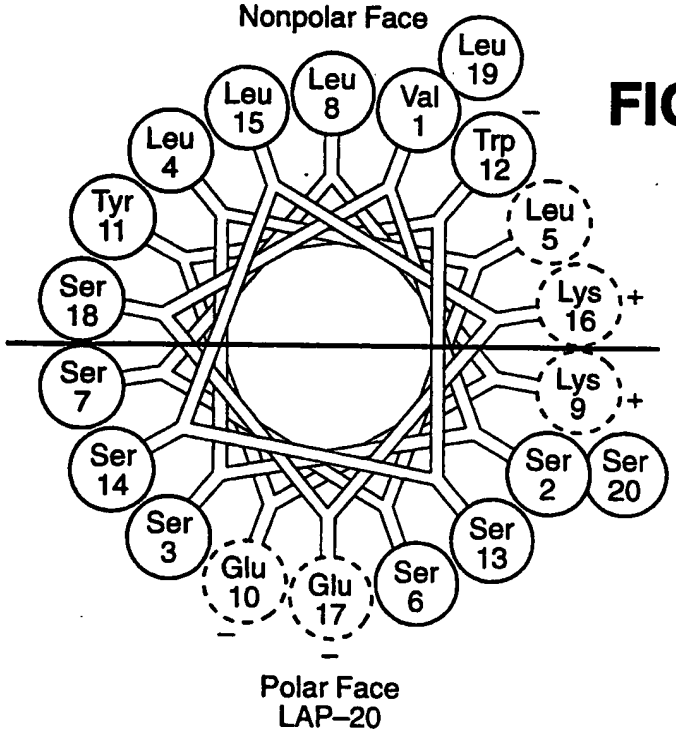
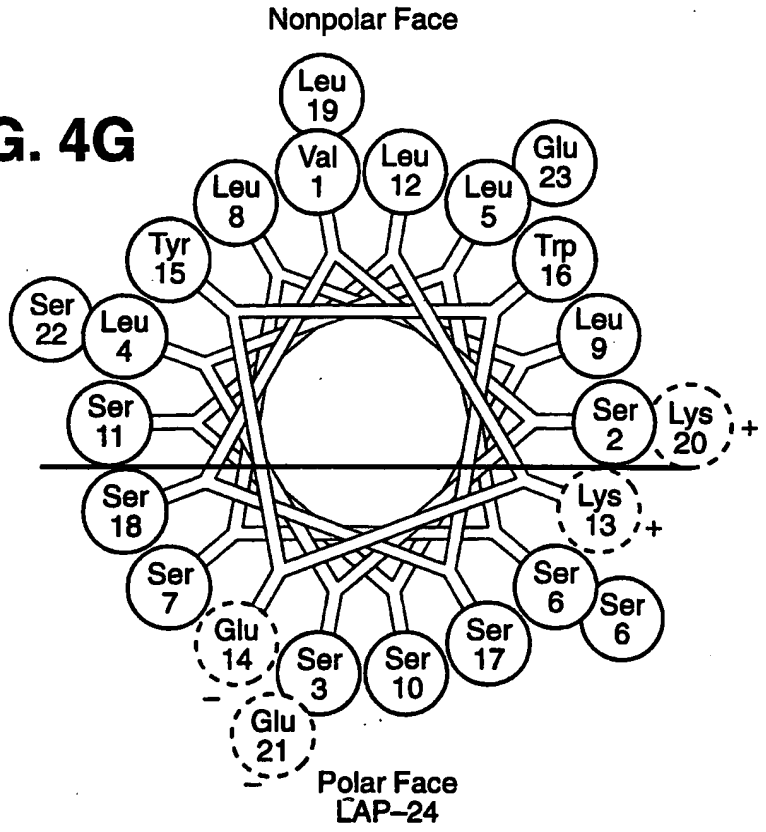

Nonpolar Face
Leu 19
Leu 15
Leu 8
Val 1
−
Leu 4
Trp 12
Tyr 11
Leu 5
Ser 18
Lys 16
+
Ser 7
Lys 9
+
Ser 14
Ser 2
Ser 20
Ser 3
Ser 13
Glu 10
Glu 17
Ser 6
−
−
Polar Face
LAP–20

FIG. 4G


Nonpolar Face
Leu 19
Val 1
Leu 12
Leu 5
Glu 23
Leu 8
Tyr 15
Trp 16
Ser 22
Leu 4
Leu 9
Ser 11
Ser 2
Lys 20
Ser 18
Lys 13
Ser 7
Ser 6
Ser 6
Glu 14
Ser 3
Ser 10
Ser 17
Glu 21
Polar Face
LAP-24

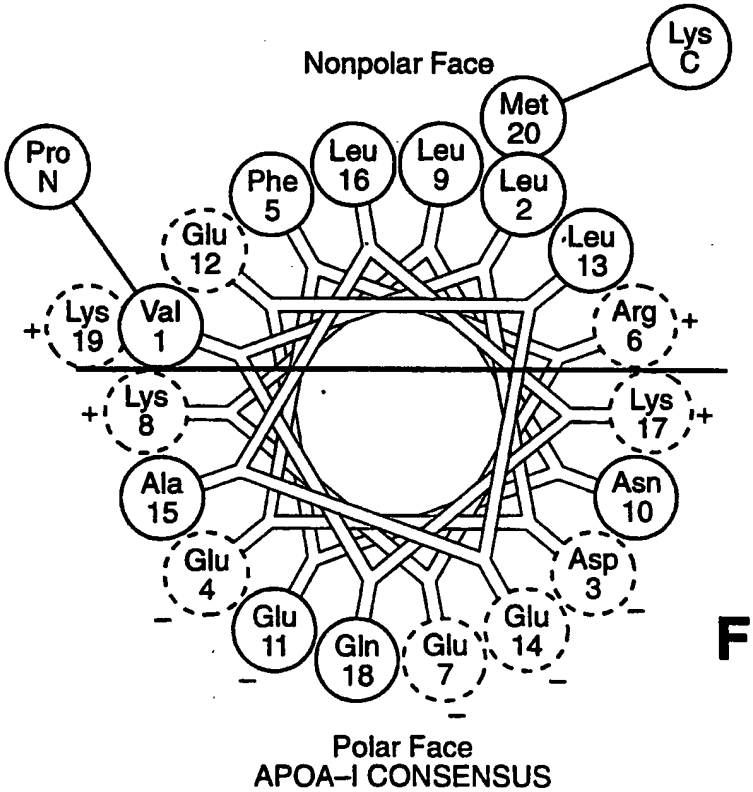
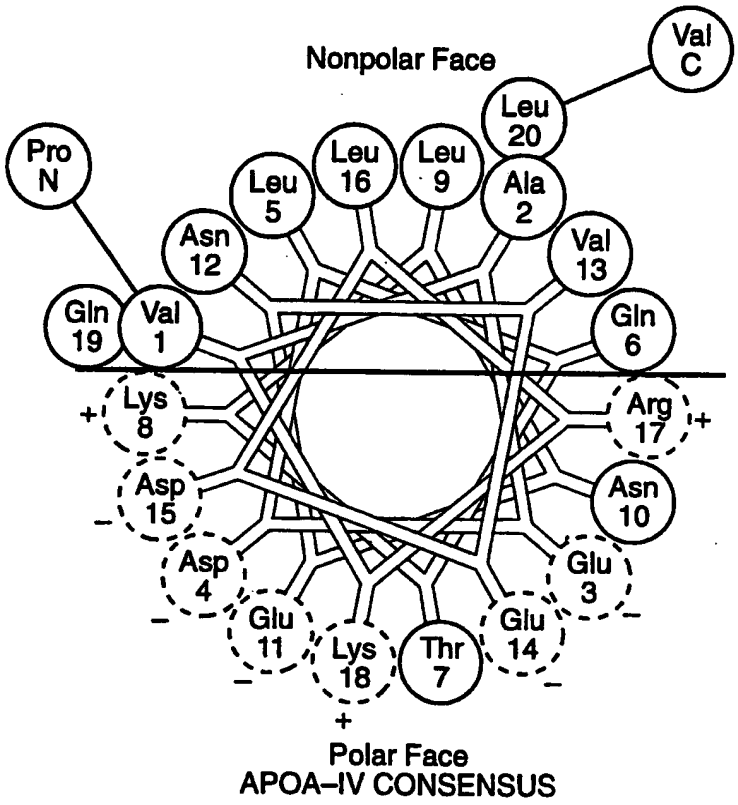

Nonpolar Face
Lys C
Met 20
Pro N
Leu 16
Leu 9
Phe 5
Leu 2
Glu 12
Leu 13
Lys 19
Val 1
Arg 6
Lys 8
Lys 17
Ala 15
Asn 10
Glu 4
Asp 3
Glu 11
Gln 18
Glu 7
Glu 14
Polar Face
APOA-I CONSENSUS

FIG. 4H

FIG. 4I


Nonpolar Face
Val C
Leu 20
Pro N
Leu 16
Leu 9
Leu 5
Ala 2
Asn 12
Val 13
Gln 19
Val 1
Gln 6
Lys 8
Arg 17
Asp 15
Asn 10
Asp 4
Glu 3
Glu 11
Lys 18
Thr 7
Glu 14
Polar Face
APOA–IV CONSENSUS

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07453

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/02; C07K 7/08, 7/10
US CL :514/2, 12, 13
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 12, 13

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US A 5,128,318 (Levine et al) 07 July 1992 See claims.	1-29
Y	Methods in Enzymology, Volume 128, issued 1986, "Synthetic Peptide Analogs of Apolipoproteins", pages 627-647. See page 630.	1-29

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 09 OCTOBER 1993	Date of mailing of the international search report 26 OCT 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer Lester L. Lee LESTER L. LEE Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

International application No.
PCT/US93/07453

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CA, Medline, Intelligenetics
Sequence ID #1
Endotoxin, Coli endotoxin tymphimurium